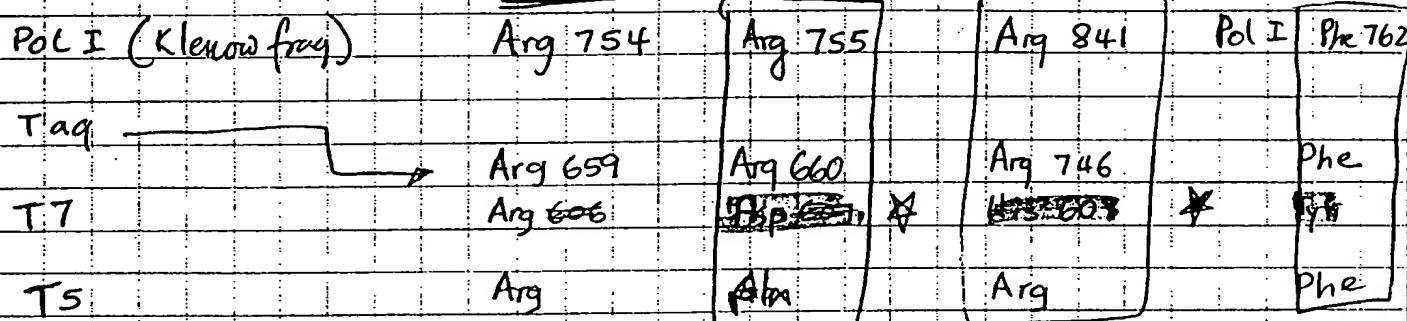


Page No. \_\_\_\_\_

The plan was to make Tag DNA pol mutant so that it can utilize deoxy or dideoxy nucleotides more effectively. This will be similar to T5 DNA pol mutation.

Comparison of the amino acid sequence of polymerases such as T5, Pol I, T7 and Tag showed some distinctive differences in and around dNTP binding sites. Note that T7 utilizes dNTPs/didNTPs equally well.

The dNTPs binds adjacent to  $\alpha$ -helix of Klenow fragment ( ). These following amino acids may be important

In  $\beta$ -helix

Pol I 677 = Gln (Q)

T5 = Gln (Q)

Tag 582 = Gln (Q)

T5 ~~Ala (A)~~ $\beta$ -helix

Pol 628 = Leu (L)

T5 = Leu

Tag = Leu

~~Tag~~ = Lys (K) $\beta$ -sheet 14

Pol 919 Gly (G)

Tag Gly (G)

T5 ~~Lys (K)~~

T5 Asp (D)

 $\alpha$ -helix in addition to Arg 841

Pol 854 Asp (D)

Tag Asp (D)

~~Tag~~ Leu (L)

T5 Asp (D)

To Page No. \_\_\_\_\_

Assed &amp; Understood by me,

Date

Invented by

Date

Recorded by

*John S. Miller*